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In this project, we stained for LRAT in normal vs malignant human breast tissues. Also, transgenic mice were generated in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively. The effects of ectopic LRAT LRAT expression on epithelial cell proliferation and differentiation were examined in these transgenic mice. We showed that with a normal level of retinol in the diet, the overexpression of human LRAT in the suprabasal layer or basal layer of the mouse epithelial cells affects mouse epithelial cell differentiation minimally. However, the epidermal hyperplasia induced by topical retinol treatment was greatly reduced in the human LRAT-expressing animals. These data suggest a role for LRAT in the maintenance of normal epithelial cells differentiation. Currently, studies are underway to assess the functions of LRAT in breast carcinoma prevention.

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Introduction

Mechanism of Action of Retinoids

Vitamin A (retinol) and its physiologically active metabolites (retinoids) play essential roles in physiological processes such as embryonic development, proliferation, differentiation, pattern formation, and apoptosis. Retinoids have major effects on the growth and differentiation of normal, premalignant, and malignant epithelial cells (Gudas et al. 1994). Vitamin A deficiency in animal models is associated with altered mammary gland differentiation and increased susceptibility to carcinogens(Metz et al. 2002). Retinoids have proven to be effective in suppressing breast cancer development in experimental models of carcinogenesis (Lotan 1980). In addition to breast cancer, retinoids have been shown to be effective in suppressing tumor development and/or treatment of cancers of the skin, oral cavity, lung, prostate, bladder, liver, and pancreas (Kelloff et al. 1996; Lotan 1996). The biological effects of retinoids are thought to be mainly mediated through interactions with retinoic acid receptors (RARs) or retinoic X receptors (RXRs), which function as dimeric transcription factors modulating gene expression by binding to RA-response elements (RAREs or RXREs) in the promoter regions of target genes (Chambon 1996).

Since retinoid receptor mediated transcription is governed at least in part by the relative abundance of receptor ligands, the ability of cells to generate bioactive intracellular retinoids may play a key role in controlling the growth and differentiation of cells. Retinol (ROL), which must be taken up by epithelial cells from the blood, is esterified within cells by the enzyme lecithin:retinol acyltransferase (LRAT) (Barry et al. 1989) **(Fig.1)**. LRAT plays an essential role in vitamin A metabolism and is particularly important in the processing of retinoids in the vertebrate visual cycle (Rando 2001). Although another enzyme, acetylCoA:retinol acyltransferase (ARAT), can esterify ROL, the enzyme responsible for the majority of ROL esterification in epithelial cells is LRAT (Herr et al. 1991). Retinyl esters (REs) are thought to be the storage form of ROL, and these esters can be gradually metabolized back into ROL by retinyl ester hydrolases (REH) within the epithelial cells.

Retinoid metabolism is disrupted in breast cancer cells. There are greatly reduced levels of REs in the carcinogen induced rat mammary carcinoma model (Bhat and Moudgal 1989). In contrast, normal breast epithelium contains extremely high levels of REs in this animal model. Our laboratory has demonstrated that the levels of REs in human carcinoma cell lines from the breast, oral cavity, kidney, and prostate were also very low (Chen et al. 1997; Guo and Gudas 1998; Guo et al. 2001; Guo et al. 2002). In addition, we demonstrated that this low level of ROL esterification was associated with the absence of LRAT enzymatic activity, the lack of detectable LRAT protein, and abnormal *LRAT* transcripts (Guo et al. 2000). Furthermore, we demonstrated that high levels of ROL and REs were detected in normal kidney epithelial tissue, but were barely detectable in kidney tumor from patients (Guo et al. 2001). More recently, we have shown that human kidney cancers which exhibited a high level of LRAT staining by immunocytochemistry were of low malignant potential, whereas those which did not stain for LRAT exhibited much higher malignant potential (Zhan 2003). These examples confirm the importance of LRAT in the maintenance of normal cell phenotype.When the cancer cells are retinoid deficient, then retinoid signaling will be compromised. For

example, the expression of RARs will be affected given that retinoids directly regulate *RARα* and *RARβ* expression (Giguere 1994). Reduction of RAR expression will further affect retinoid signaling (Boylan et al. 1995; Isogai et al. 1997). This is particularly important as the growth inhibitory effects of retinoids are mediated largely by *RARα* and *RARβ (*van der Burg et al. *1993)*. Thus, a deficiency in esterification activity would lead to improper retinoid signaling and could contribute to carcinoma formation.

The goal of this proposal is to determine the contribution of esterification of ROL to breast cancer growth inhibition and tumor inhibition. We will determine whether LRAT- mediated esterification of ROL can enhance the retinoid signaling in tumor cells and decrease breast cancer cell growth. The results from these studies could provide a rationale for targeting LRAT or the ROL esterification process in novel preventive and therapeutic treatments for breast cancer patients.

In this project, we stained for LRAT in normal vs malignant human breast tissues. Also, transgenic mice were generated in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively. The effects of ectopic LRAT LRAT expression on epithelial cell proliferation and differentiation were examined in these transgenic mice. We showed that with a normal level of retinol in the diet, the overexpression of human LRAT in the suprabasal layer or basal layer of the mouse epithelial cells affects mouse epithelial cell differentiation minimally. However, the epidermal hyperplasia induced by topical retinol treatment was greatly reduced in the human LRAT-expressing animals. These data suggest a role for LRAT in the maintenance of normal epithelial cells differentiation. Currently, studies are underway to assess the functions of LRAT in breast carcinoma prevention.

Body

Part 1 Measurement of LRAT in Normal Human Breast Tissue vs Tissue from Breast Cancer Patients

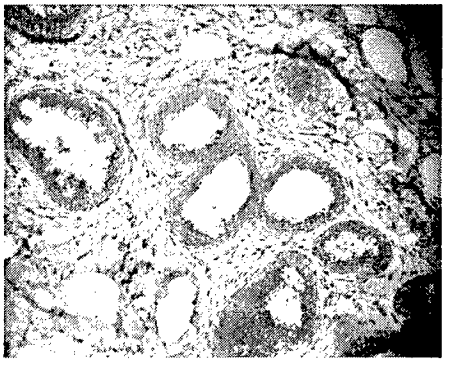

Figure 1. A sample from a human breast cancer patient (NY Hospital/Cornell) with normal ducts showing LRAT expression (brown stain) in the luminal cells. The surrounding stroma does not stain for LRAT. (Blue – nuclear stain in all cells). Tumor tissue from the same patient does not stain for LRAT (not shown).

Part 2: Generation of transgenic mice in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively.

1. Construction of transgenes

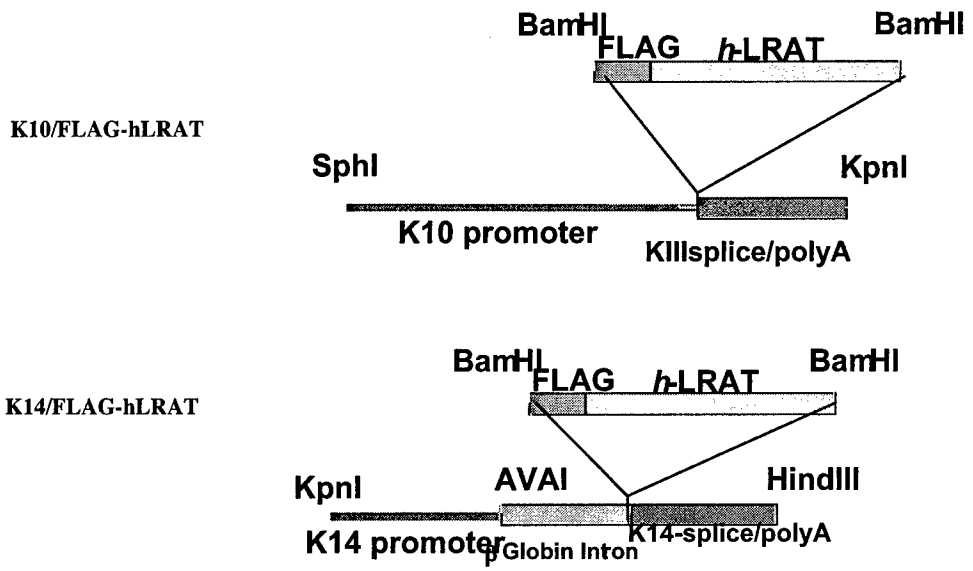

Fig 2. Constructions of transgenes

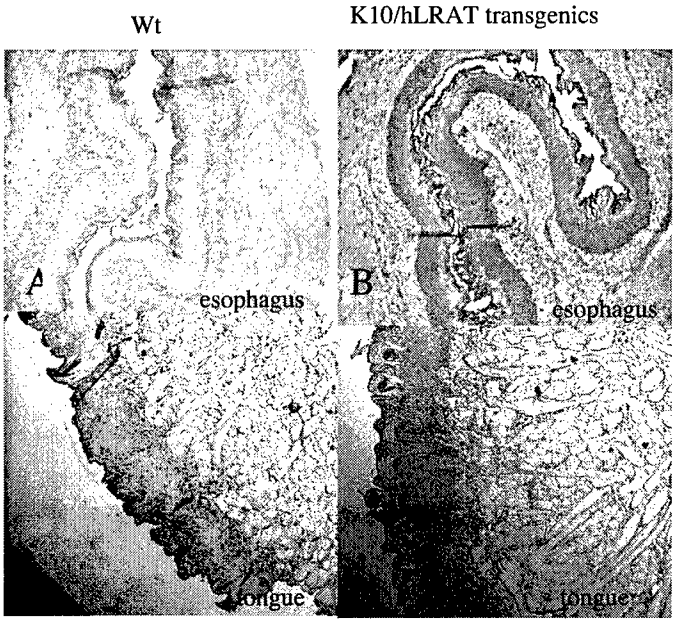

Fig 3. Expression of hLRAT in the suprabasal layer of keratinocytes. Sections from mouse tongue and esophagus were paraffin-embedded, immunostained with anti-hLRAT antibody, and the stain was developed by DAB. (A,C, wild-type mice; B,D, K10/hLRAT transgenics)

2. Topical retinol or RA treatment in wild type and hLRAT transgenic mice

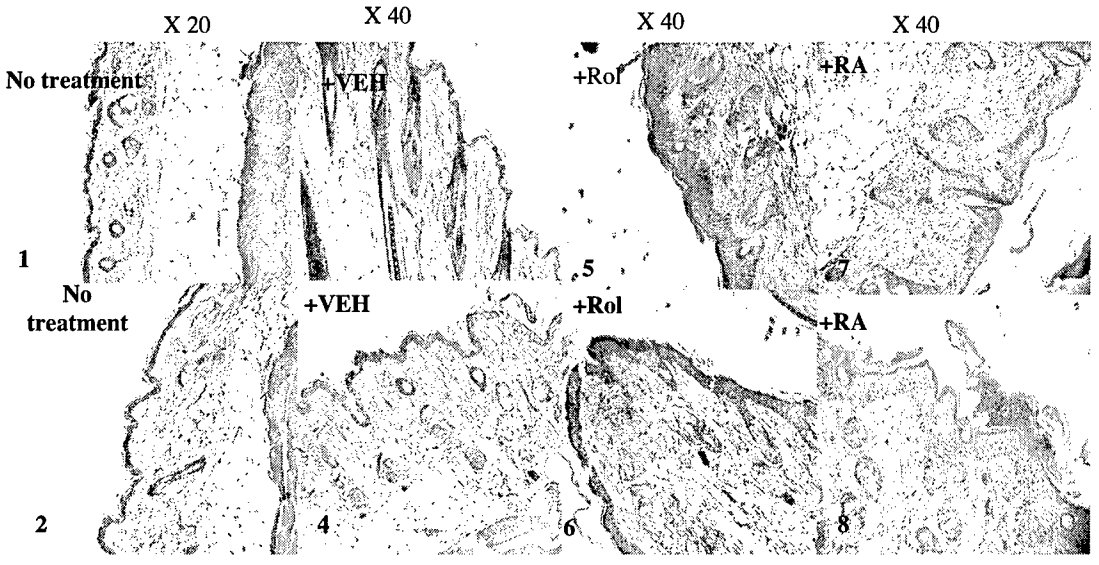

Fig 4a. Reduced epidermal response to *all-trans* retinol in *h*LRAT expressing mice. Micrographs of sections of mouse dorsal skin after topical treatment with retinoids. 400 _l of retinoids (RA or Rol 0.1mM) in acetone (Vehicle), were applied to the dorsal skin (2 x 3 cm^2) of transgenic mice or their wild-type littermates. After 4-day topical treatments, skin was paraffin- embedded and H&E stained. 1,3,5,7: Wild type ; 2,4,6,8: K10-31 hLRAT+ transgenics.

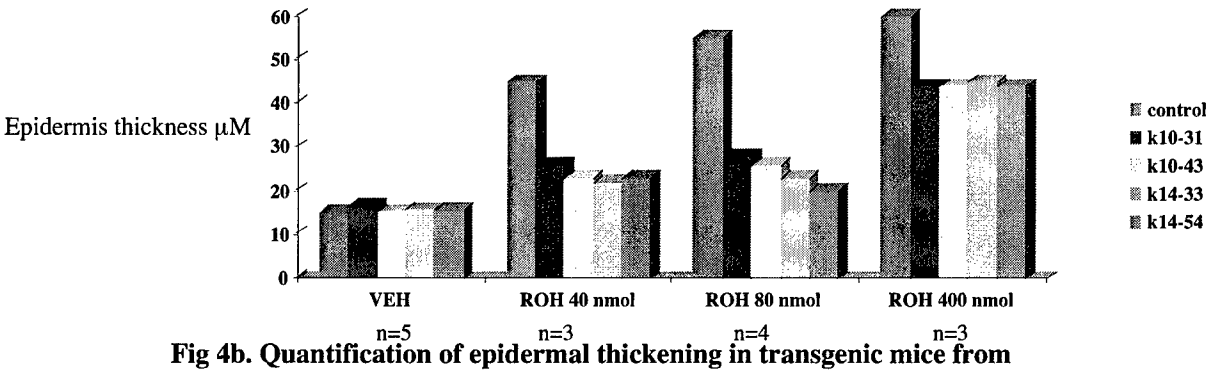

Fig 4b. Quantification of epidermal thickening in transgenic mice from 4 independent lines after topical retinol treatment. 5 different areas per section were randomly picked and measured under microscope.

3. Retinol metabolism after topical retinol or RA treatments in wild type and hLRAT transgenic mice

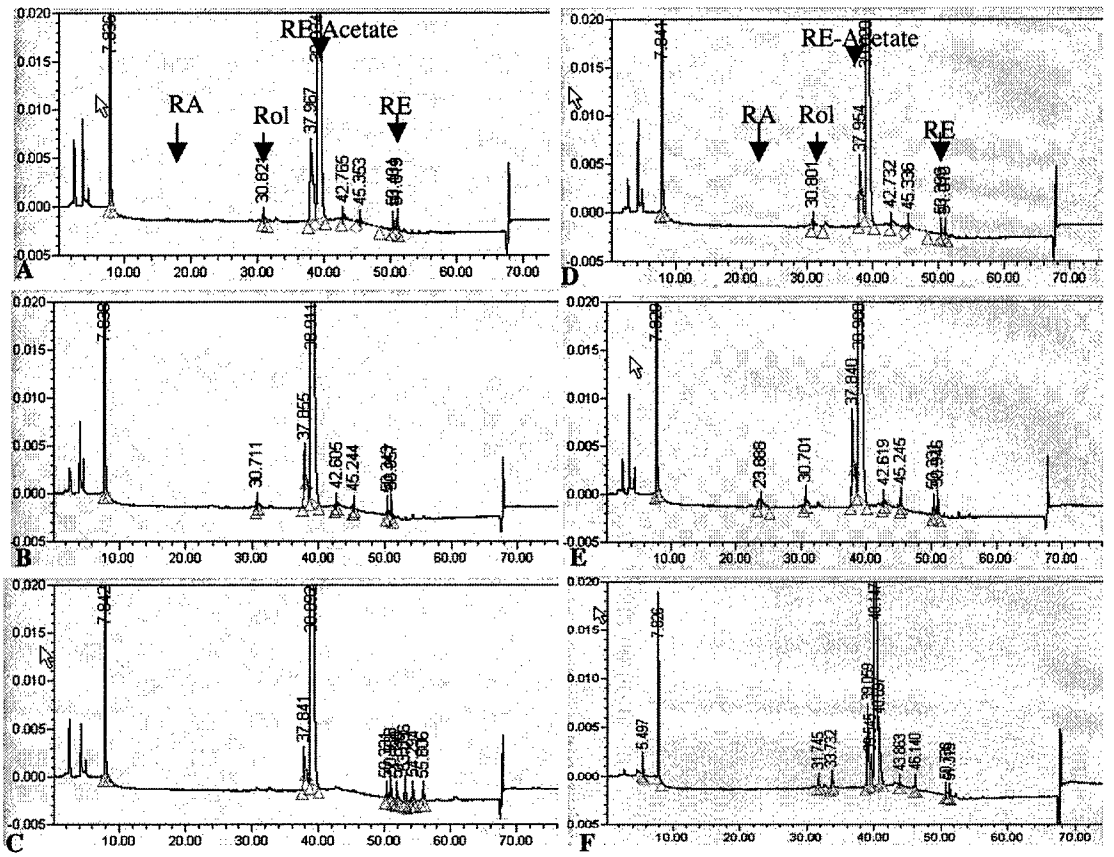

Fig 5. Higher retinyl–ester synthesis in K10-hLRAT transgenic mice than in wild-type mice. Epidermal retinoids in k10-hLRAT transgenic mice and wild-type littermates were detected by High Performance Liquid Chromatography (HPLC) as described by Guo etal., 2001. After a 4-day topical treatment with retinoids (40 nmol in 400 uL daily) or vehicle (acetone) to the mouse back skin, 0.05g of back skin was processed. A, Wt mouse treated with acetone; B, Wt mouse treated with RA; C, Wt mouse treated Rol; D, K10-31 transgenic mouse treated with acetone; E, K10-31 transgenic mouse treated with RA; F, K10-31 transgenic mouse treated Rol. The retinoids corresponding to the respective retention times are indicated by arrows. Retinyl acetate, an internal control, was added to the skin tissue homogenates.

Key research accomplishments

1. There is reduced *all-trans* retinol (ROL) -induced epidermal hyperplasia in k10-flag*h*LRAT expressing mice.
2. Expression of *h*LRAT in suprabasal keratinocytes reduces ROL but not RA's stimulatory effect on keratinocyte proliferation.
3. HPLC studies showed that epidermis from suprabasal hLRAT expressing mice esterify more retinol after the retinol topical treatments than the wild- type mice.

4. Our results indicate that ectopic LRAT-mediated retinol metabolism controls aspects of epithelial cell growth and differentiation.

Reportable Outcomes

Su D and Gudas LJ. Ectopic expression of human lecithin:retinol acyltransferase in mouse suprabasal epithelial cells results in resistance of the skin to topical all-*trans* retinol induced epidermal hyperplasia (Manuscript in preparation)

Shren, M., Su, D., Bok D.,Gallagher,L.and Gudas LJ. LRATstaining in Normal and Tumor Tissue from Human Breast Cancer Patients (Manuscript in preparation)

Conclusions

Transgenic mice were generated in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively. The effects of ectopic LRAT LRAT expression on epithelial cell proliferation and differentiation were examined in these transgenic mice. We showed that with normal level of retinol in the diet, the overexpression of human LRAT in the suprabasal layer or basal layer of the mouse epithelial cells affects mouse epithelial cells differentiation minimally. However, the epidermal hyperplasia induced by topical retinol treatment was greatly reduced in the human LRAT-expressing animals. These data suggest a role for LRAT in the maintenance of normal epithelial cells differentiation. Currently, studies are underway to assess the functions of LRAT in breast carcinoma prevention.

References

Barry, R.J., F.J. Canada, and R.R. Rando. 1989. Solubilization and partial purification of retinyl ester synthetase and retinoid isomerase from bovine ocular pigment epithelium. *J Biol Chem* **264**: 9231-8.

Bhat, P.J. and N.R. Moudgal. 1989. Isolation and characterization of a gonadotropin receptor binding inhibitor from porcine follicular fluid. *Int J Pept Protein Res* **33**: 59-66.

Boylan, J.F., T. Lufkin, C.C. Achkar, R. Taneja, P. Chambon, and L.J. Gudas. 1995. Targeted disruption of retinoic acid receptor alpha (RAR alpha) and RAR gamma results in receptor-specific alterations in retinoic acid-mediated differentiation and retinoic acid metabolism. *Mol Cell Biol* **15**: 843-51.

Chambon, P. 1996. A decade of molecular biology of retinoic acid receptors. *FASEB J.* **10**: 940-954.

Chen, A.C., X. Guo, F. Derguini, and L.J. Gudas. 1997. Human breast cancer cells and normal mammary epithelial cells: retinol metabolism and growth inhibition by the retinol metabolite 4-oxoretinol. *Cancer Res* **57**: 4642-51.

Giguere, V. 1994. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. *Endocr Rev* **15**: 61-79.

Gudas, L.J. 1994. Retinoids and vertebrate development. *J. Biol. Chem.* **269**: 15399-15402.

Gudas, L.J., M.B. Sporn, and A.B. Roberts. 1994. Cellular biology and biochemistry of the retinoids. In *The Retinoids: Biology, Chemistry, and Medicine* (ed. M.B. Sporn, A.B. Roberts, and D.S. Goodman), pp. 443-520. Raven Press, New York.

Guo, X. and L.J. Gudas. 1998. Metabolism of all-*trans*-retinol in normal human cell strains and squamous cell carcinoma lines from the oral cavity and skin: reduced esterification of retinol in SCC lines. *Cancer Res.* **58**: 166-176.

Guo, X., B.S. Knudsen, D.M. Peehl, A. Ruiz, D. Bok, R.R. Rando, J.S. Rhim, D.M. Nanus, and L.J. Gudas. 2002. Retinol metabolism and lecithin:retinol acyltransferase levels are reduced in cultured human prostate cancer cells and tissue specimens. *Cancer Res* **62**: 1654-61.

Guo, X., D.M. Nanus, A. Ruiz, R.R. Rando, D. Bok, and L.J. Gudas. 2001. Reduced levels of retinyl esters and vitamin A in human renal cancers. *Cancer Res.* **61**: 2774-2781.

Guo, X., A. Ruiz, R.R. Rando, D. Bok, and L.J. Gudas. 2000. Esterification of all-trans-retinol in normal human epithelial cell strains and carcinoma lines from oral cavity, skin and breast: reduced expression of lecithin:retinol acyltransferase in carcinoma lines. *Carcinogenesis* **21**: 1925-33.

Herr, F.M., P.N. MacDonald, and D.E. Ong. 1991. Solubilization and partial characterization of lecithin-retinol acyltransferase from rat liver. *J. Nutr. Biochem.* **2**: 503-511.

Isogai, M., M.V. Chiantore, M. Haque, G. Scita, and L.M. De Luca. 1997. Expression of a dominant-negative retinoic acid receptor construct reduces retinoic acid metabolism and retinoic acid-induced inhibition of NIH-3T3 cell growth. *Cancer Res* **57**: 4460-4.

Kelloff, G.J., C.W. Boone, J.A. Crowell, V.E. Steele, R.A. Lubet, L.A. Doody, W.F. Malone, E.T. Hawk, and C.C. Sigman. 1996. New agents for cancer chemoprevention. *J Cell Biochem Suppl* **26**: 1-28.

Lotan, R. 1980. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. *Biochim Biophys Acta* **605**: 33-91.

-. 1996. Retinoids in cancer chemoprevention. *FASEB J.* **10**: 1031-1039.

Mangelsdorf, D.J., C. Thummel, M. Beato, P. Herrlich, G. Schütz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, and R.M. Evans. 1995. The nuclear receptor superfamily: the second decade. *Cell* **83**: 835-840.

Metz, R.P., M. Kaeck, M. Stacewicz-Sapuntzakis, T. Mitrenga, H. McCarty, and P. Schedin. 2002. Adolescent vitamin A intake alters susceptibility to mammary carcinogenesis in the Sprague-Dawley rat. *Nutr Cancer* **42**: 78-90.

Rando, R.R. 2001. The biochemistry of the visual cycle. *Chem Rev* **101**: 1881-96.

Sun, S.Y. and R. Lotan. 2002. Retinoids and their receptors in cancer development and chemoprevention. *Crit. Rev. Oncol. Hematol.* **41**: 41-55.

Tallman, M.S. and C. Nabhan. 2002. Management of acute promyelocytic leukemia. *Curr Oncol Rep* **4**: 381-9.

van der Burg, B., B.-J. van der Leede, L. Kwakkenbos-Isbrücker, S. Salverda, S.W. de Laat, and P.T. van der Saag. 1993. Retinoic acid resistance of estradiol-independent breast cancer cells coincides with diminished retinoic acid receptor function. *Mol. Cell. Endo.* **91**: 149-157.

Zhan, H., Gudas, LJ., Bok, D., Rando, R., Nanus, DM., Tickoo, SK. 2003. Differential expresssion of the enzyme which esterifies retinol, lecithin:retinol acyltransferase, in subtyps of human renal cancer and normal kidney. *Clinical Cancer Research (in press).*